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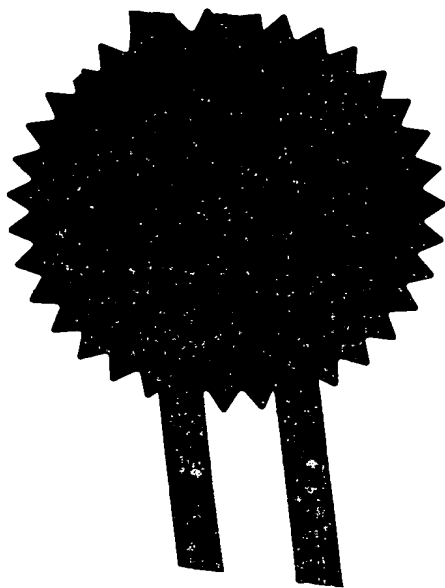
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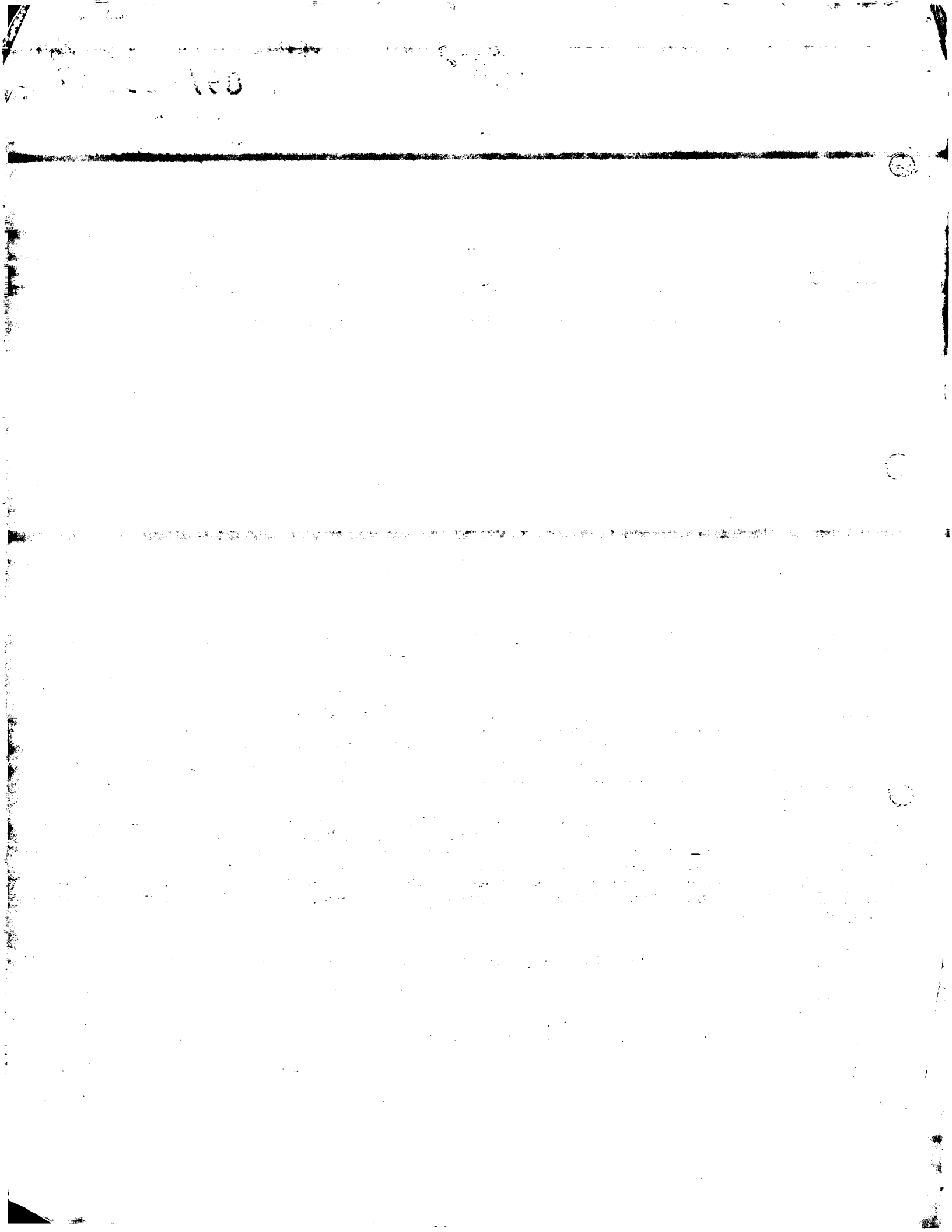
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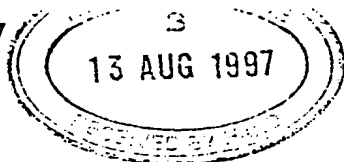
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Patents ADP number *(if you know it)*

6857242 001

If the applicant is a corporate body, give the
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UNITED KINGDOM

4. Title of the invention

GENETIC CONTROL OF PLANT GROWTH AND DEVELOPMENT

5. Name of your agent *(if you have one)*

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GENETIC CONTROL OF PLANT GROWTH AND DEVELOPMENT

This invention relates to the genetic control of growth and/or development of plants and the cloning and expression of genes involved therein. More particularly, the invention

5   relates to the cloning and expression of the *Rht* gene of *Triticum Aestivum*, and homologues from other species, and use of the genes in plants.

An understanding of the genetic mechanisms which influence growth and development of plants, including

10   flowering, provides a means for altering the characteristics of a target plant. Species for which manipulation of growth and/or development characteristics may be advantageous includes all crops, with important examples being the cereals, rice and maize, probably the most agronomically important in

15   warmer climatic zones, and wheat, barley, oats and rye in more temperate climates. Important crops for seed products are oil seed rape and canola, maize, sunflower, soyabean and sorghum. Many crops which are harvested for their roots are, of course, grown annually from seed and the production of seed of any

20   kind is very dependent upon the ability of the plant to flower, to be pollinated and to set seed. In horticulture, control of the timing of growth and development, including flowering, is important. Horticultural plants whose flowering may be controlled include lettuce, endive and vegetable

25   brassicas including cabbage, broccoli and cauliflower, and carnations and geraniums. Dwarf plants on the one hand and over-size, taller plants on the other may be advantageous and/or desirable in various horticultural and agricultural

contexts, further including trees, plantation crops and grasses.

Recent decades have seen huge increases in wheat grain yields due to the incorporation of semi-dwarfing *Rht*
5 homeoalleles into breeding programmes. These increases have enabled wheat productivity to keep pace with the demands of the rising world population. Previously, we described the cloning of the *Arabidopsis gai* alleles (unpublished International patent application PCT/GB97/00390 filed 12
10 February 1997, John Innes Centre Innovations Limited, the full contents of which are incorporated herein by reference) which, like *Rht* mutant alleles, confers a semi-dominant dwarf phenotype and a reduction in responsiveness to the plant growth hormone gibberellin (GA). *gai* encodes a mutant protein
15 (gai) which lacks a 17 amino acid residue segment found near the N-terminus of the wild-type (GAI) protein. The sequence of this segment is highly conserved in a rice cDNA sequence (EST). Here we show that this cDNA maps to a short section of the overlapping cereal genome maps known to contain the *Rht*
20 loci, and that we have used the cDNA to isolate the *Rht* genes of wheat. That genomes as widely diverged as those of *Arabidopsis* and *Triticum* should carry a conserved sequence which, when mutated, affects GA responsiveness, indicates a role for that sequence in GA signalling that is conserved
25 throughout the plant kingdom. Furthermore, cloning of *Rht* permits its transfer to many different crop species, with the aim of yield enhancement as great as that obtained previously with wheat.

The introduction of semi-dwarfing *Rht* homeoalleles
(originally known as Norin 10 genes, derived from a Japanese
variety, Norin 10) into elite bread-wheat breeding lines was
one of the most significant contributors to the so-called

5 "green revolution" (Gale *et al*, 1985. Dwarfing genes in
wheat. In: Progress in Plant Breeding, G.E. Russell (ed)
Butterworths, London pp 1-35). Wheat containing these
homeoalleles consistently out-yield wheats lacking them, and
now comprise around 80% of the world's wheat crop. The

10 biological basis of this yield-enhancement appears to be two-
fold. Firstly, the semi-dwarf phenotype conferred by the *Rht*
alleles causes an increased resistance to lodging (flattening
of plants by wind/rain with consequent loss of yield).
Secondly, these alleles cause a reallocation of

15 photoassimilate, with more being directed towards the grain,
and less towards the stem (Gale *et al*, 1985). These
properties have major effects on wheat yields, as demonstrated
by the fact that UK wheat yields increased by over 20% during
the years that *Rht*-containing lines were taken up by farmers.

20 The *rht* mutants are dwarfed because they contain a
genetically dominant, mutant *rht* allele which compromises
their responses to gibberellin (GA, an endogenous plant growth
regulator) (Gale *et al*, 1976. Heredity 37; 283-289). Thus
the coleoptiles of *rht* mutants, unlike those of wild-type

25 wheat plants, do not respond to GA applications. In addition,
rht mutants accumulate biologically active GAs to higher
levels than found in wild-type controls (Lenton *et al*, 1987.
Gibberellin insensitivity and depletion in wheat -

4

consequences for development. In: Hormone action in Plant
Development - a critical appraisal. GV Haod, JR Lenton, MB
Jackson and RK Atkin (eds) Butterworths, London pp 145-160).
These properties (genetic dominance, reduced GA-responses, and
5 high endogenous GA levels) are common to the phenotypes
conferred by mutations in other species (*D8/D9* in maize; *gai*
in *Arabidopsis*), indicating that these mutant alleles define
orthologous genes in these different species, supported
further by the observation that *D8/D9* and *Rht* are syntenous
10 loci in the genomes of maize and wheat.

According to a first aspect of the present invention
there is provided a nucleic acid molecule comprising a
nucleotide sequence encoding a polypeptide with Rht function.
15 The term "Rht function" indicates ability to influence the
phenotype of a plant like the *Rht* gene of *Triticum*. "*Rht*
function" may be observed phenotypically in a plant as
inhibition, suppression, repression or reduction of plant
growth which inhibition, suppression, repression or reduction
20 is antagonised by GA. *Rht* expression tends to confer a dwarf
phenotype on a plant which is antagonised by GA.
Overexpression in a plant from a nucleotide sequence encoding
a polypeptide with *Rht* function may be used to confer a dwarf
phenotype on a plant which is correctable by treatment with
25 GA.
Also according to an aspect of the present invention
there is provided a nucleic acid molecule comprising a
nucleotide sequence encoding a polypeptide with ability to

confer a *rht* mutant phenotype upon expression. *rht* mutant
plants are dwarfed compared with wild-type, the dwarfing being
GA-insensitive.

Herein, "Rht" (capitalised) is used to refer to the wild-
5   type function, while "rht" (uncapitalised) is used to refer to
mutant function.

Many plant growth and developmental processes are
regulated by specific members of a family of tetracyclic
diterpenoid growth factors known as gibberellins (GA) (Hooley,
10   *Plant Mol. Biol.* 26, 1529-1555 (1994)). By gibberellin or GA
is meant a diterpenoid molecule with the basic carbon-ring
structure shown in Figure 6 and possessing biological
activity, i.e. we refer to biologically active gibberellins.

Biological activity may be defined by one or more of
15   stimulation of cell elongation, leaf senescence or elicitation
of the cereal aleurone α-amylase response. There are many
standard assays available in the art, a positive result in any
one or more of which signals a test gibberellin as
biologically active (Hoad et al., *Phytochemistry* 20, 703-713
20   (1981); Serebryakov et al., *Phytochemistry* 23, 1847-1854
(1984); Smith et al., *Phytochemistry* 33, 17-20 (1993)).

Assays available in the art include the lettuce hypocotyl
assay, cucumber hypocotyl assay, and oat first leaf assay, all
of which determine biological activity on the basis of ability
25   of an applied gibberellin to cause elongation of the
respective tissue. Preferred assays are those in which the
test composition is applied to a gibberellin-deficient plant.
Such preferred assays include treatment of dwarf GA-deficient

Arabidopsis to determine growth, the dwarf pea assay, in which internode elongation is determined, the Tan-ginbozu dwarf rice assay, in which elongation of leaf sheath is determined, and the *d5*-maize assay, also in which elongation of leaf sheath is

5   determined. The elongation bioassays measure the effects of general cell elongation in the respective organs and are not restricted to particular cell types.

Further available assays include the dock (*Rumex*) leaf senescence assay and the cereal aleurone α-amylase assay.

10 Aleurone cells which surround the endosperm in grain secrete α-amylase on germination, which digests starch to produce sugars then used by the growing plant. The enzyme production is controlled by GA. Isolated aleurone cells given biologically active GA secrete α-amylase whose activity can

15 then be assayed, for example by measurement of degradation of starch.

Structural features important for high biological activity (exhibited by GA_1, GA_3, GA_4 and GA_7) are a carboxyl group on C-6 of B-ring; C-19, C-10 lactone; and β-

20 hydroxylation at C-3. β-hydroxylation at C-2 causes inactivity (exhibited by GA_8, GA_{29}, GA_{34} and GA_{51}). *rht* mutants do not respond to GA treatment, e.g. treatment with GA_1, GA_3 or GA_4.

Treatment with GA is preferably by spraying with aqueous

25 solution, for example spraying with 10^{-4}M GA_3 or GA_4 in aqueous solution, perhaps weekly or more frequently, and may be by placing droplets on plants rather than spraying. GA may be applied dissolved in an organic solvent such as ethanol or

acetone, because it is more soluble in these than in water, but this is not preferred because these solvents have a tendency to damage plants. If an organic solvent is to be used, suitable formulations include $24\eta l$ of 0.6, 4.0 or 300mM

5 GA_3 or GA_4 dissolved in 80% ethanol. Plants, e.g. *Arabidopsis*, may be grown on a medium containing GA, such as tissue culture medium (GM) solidified with agar and containing supplementary GA.

Nucleic acid according to the present invention may have

10 the sequence of a wild-type *Rht* gene of *Triticum* or be a mutant, derivative, variant or allele of the sequence provided. Preferred mutants, derivatives, variants and alleles are those which encode a protein which retains a functional characteristic of the protein encoded by the wild-

15 type gene, especially the ability for plant growth inhibition, which inhibition is antagonised by GA, or ability to confer on a plant one or more other characteristics responsive to GA treatment of the plant. Other preferred mutants, derivatives, variants and alleles encode a protein which confers a *rht*

20 mutant phenotype, that is to say reduced plant growth which reduction is insensitive to GA, i.e. not overcome by GA treatment. Changes to a sequence, to produce a mutant, variant or derivative, may be by one or more of addition, insertion, deletion or substitution of one or more nucleotides

25 in the nucleic acid, leading to the addition, insertion, deletion or substitution of one or more amino acids in the encoded polypeptide. Of course, changes to the nucleic acid which make no difference to the encoded amino acid sequence

are included.

A preferred nucleotide sequence for a *Rht* gene is one which encodes the RHT amino acid sequence shown in Figure 3b, especially a *Rht* coding sequence shown in Figure 3a. A

5 preferred *rht* mutant lacks part or all of the 17 amino acid sequence underlined in Figure 3b, and/or part or the sequence DVAQKLEQLE, which immediately follows the 17 amino acid sequence underlined in Figure 3b.

The present invention also provides a nucleic acid

10 construct or vector which comprises nucleic acid with any one of the provided sequences, preferably a construct or vector from which polypeptide encoded by the nucleic acid sequence can be expressed. The construct or vector is preferably suitable for transformation into a plant cell. The invention

15 further encompasses a host cell transformed with such a construct or vector, especially a plant cell. Thus, a host cell, such as a plant cell, comprising nucleic acid according to the present invention is provided. Within the cell, the nucleic acid may be incorporated within the chromosome. There

20 may be more than one heterologous nucleotide sequence per haploid genome. This, for example, enables increased expression of the gene product compared with endogenous levels, as discussed below.

A construct or vector comprising nucleic acid according

25 to the present invention need not include a promoter or other regulatory sequence, particularly if the vector is to be used to introduce the nucleic acid into cells for recombination into the genome. However, in one aspect the present invention

provides a nucleic acid construct comprising a *Rht* or *rht* coding sequence (which includes homologues from other than *Triticum*) joined to a regulatory sequence for control of expression, the regulatory sequence being other than that

5 naturally fused to the coding sequence and preferably of or derived from another gene.

Nucleic acid molecules and vectors according to the present invention may be as an isolate, provided isolated from their natural environment, in substantially pure or

10 homogeneous form, or free or substantially free of nucleic acid or genes of the species of interest or origin other than the sequence encoding a polypeptide able to influence growth and/or development, which may include flowering, eg in *Triticum Aestivum* nucleic acid other than the *Rht* coding

15 sequence. The term "nucleic acid isolate" encompasses wholly or partially synthetic nucleic acid.

Nucleic acid may of course be double- or single-stranded, cDNA or genomic DNA, RNA, wholly or partially synthetic, as appropriate. Of course, where nucleic acid according to the

20 invention includes RNA, reference to the sequence shown should be construed as reference to the RNA equivalent, with U substituted for T.

The present invention also encompasses the expression product of any of the nucleic acid sequences disclosed and

25 methods of making the expression product by expression from encoding nucleic acid therefor under suitable conditions in suitable host cells. Those skilled in the art are well able to construct vectors and design protocols for expression and

recovery of products of recombinant gene expression. Suitable
vectors can be chosen or constructed, containing appropriate
regulatory sequences, including promoter sequences, terminator
fragments, polyadenylation sequences, enhancer sequences,

5   marker genes and other sequences as appropriate. For further
details see, for example, *Molecular Cloning: a Laboratory
Manual*: 2nd edition, Sambrook et al, 1989, Cold Spring Harbor
Laboratory Press. Transformation procedures depend on the
host used, but are well known. Many known techniques and

10   protocols for manipulation of nucleic acid, for example in
preparation of nucleic acid constructs, mutagenesis,
sequencing, introduction of DNA into cells and gene
expression, and analysis of proteins, are described in detail
in *Protocols in Molecular Biology*, Second Edition, Ausubel et

15   al. eds., John Wiley & Sons, 1992. Specific procedures and
vectors previously used with wide success upon plants are
described by Bevan, Nucl. Acids Res. (1984) 12, 8711-8721),
and Guerineau and Mullineaux, (1993) Plant transformation and
expression vectors. In: Plant Molecular Biology Labfax (Croy

20   RRD ed) Oxford, BIOS Scientific Publishers, pp 121-148. The
disclosures of Sambrook et al. and Ausubel et al. and all
other documents mentioned herein are incorporated herein by
reference.

     Expression as a fusion with a polyhistidine tag allows

25   purification of Rht or rht to be achieved using Ni-NTA resin
available from QIAGEN Inc. (USA) and DIAGEN GmbH (Germany).
See Janknecht et al., *Proc. Natl. Acad. Sci. USA* 88, 8972-8976
(1991) and EP-A-0253303 and EP-A-0282042. Ni-NTA resin has

high affinity for proteins with consecutive histidines close
to the N- or C- terminus of the protein and so may be used to
purifiy histidine-tagged Rht or rht proteins from plants,
plant parts or extracts or from recombinant organisms such as
5   yeast or bacteria, e.g. *E. coli*, expressing the protein.

Purified Rht protein, e.g. produced recombinantly by
expression from encoding nucleic acid therefor, may be used to
raise antibodies employing techniques which are standard in
the art. Antibodies and polypeptides comprising antigen-
10 binding fragments of antibodies may be used in identifying
homologues from other species as discussed further below.

Methods of producing antibodies include immunising a
mammal (eg human, mouse, rat, rabbit, horse, goat, sheep or
monkey) with the protein or a fragment thereof. Antibodies
15 may be obtained from immunised animals using any of a variety
of techniques known in the art, and might be screened,
preferably using binding of antibody to antigen of interest.
For instance, Western blotting techniques or
immunoprecipitation may be used (Armitage et al, 1992, Nature
20 357: 80-82). Antibodies may be polyclonal or monoclonal.

As an alternative or supplement to immunising a mammal,
antibodies with appropriate binding specificty may be obtained
from a recombinantly produced library of expressed
immunoglobulin variable domains, eg using lambda bacteriophage
25 or filamentous bacteriophage which display functional
immunoglobulin binding domains on their surfaces; for instance
see WO92/01047.

Antibodies raised to a Rht, or rht, polypeptide can be

used in the identification and/or isolation of homologous polypeptides, and then the encoding genes. Thus, the present invention provides a method of identifying or isolating a polypeptide with Rht function or ability to confer a *rht*

5 mutant phenotype, comprising screening candidate polypeptides with a polypeptide comprising the antigen-binding domain of an antibody (for example whole antibody or a fragment thereof) which is able to bind an *Triticum Aestivum* Rht or rht polypeptide, or preferably has binding specificity for such a

10 polypeptide, such as having the amino acid sequence shown in Figure 4.

Candidate polypeptides for screening may for instance be the products of an expression library created using nucleic acid derived from an plant of interest, or may be the product

15 of a purification process from a natural source.

A polypeptide found to bind the antibody may be isolated and then may be subject to amino acid sequencing. Any suitable technique may be used to sequence the polypeptide either wholly or partially (for instance a fragment of the

20 polypeptide may be sequenced). Amino acid sequence information may be used in obtaining nucleic acid encoding the polypeptide, for instance by designing one or more oligonucleotides (e.g. a degenerate pool of oligonucleotides) for use as probes or primers in hybridisation to candidate

25 nucleic acid, as discussed further below.

A further aspect of the present invention provides a method of identifying and cloning *Rht* homologues from plant species other than *Triticum* which method employs a nucleotide

sequence derived from any shown in Figure 2 or Figure 3a. Sequences derived from these may themselves be used in identifying and in cloning other sequences. The nucleotide sequence information provided herein, or any part thereof, may

5 be used in a data-base search to find homologous sequences, expression products of which can be tested for *Rht* function. Alternatively, nucleic acid libraries may be screened using techniques well known to those skilled in the art and homologous sequences thereby identified then tested.

10   For instance, the present invention also provides a method of identifying and/or isolating a *Rht* or *rht* homologue gene, comprising probing candidate (or "target") nucleic acid with nucleic acid which encodes a polypeptide with *Rht* function or a fragment or mutant, derivative or allele

15 thereof. The candidate nucleic acid (which may be, for instance, cDNA or genomic DNA) may be derived from any cell or organism which may contain or is suspected of containing nucleic acid encoding such a homologue.

In a preferred embodiment of this aspect of the present

20 invention, the nucleic acid used for probing of candidate nucleic acid encodes an amino acid sequence shown in Figure 3b, a sequence complementary to a coding sequence, or a fragment of any of these, most preferably comprising a nucleotide sequence shown in Figure 3a.

25   Alternatively, as discussed, a probe may be designed using amino acid sequence information obtained by sequencing a polypeptide identified as being able to be bound by an antigen-binding domain of an antibody which is able to bind a

Rht or rht polypeptide such as one with the Rht amino acid sequence shown in Figure 3b.

Preferred conditions for probing are those which are stringent enough for there to be a simple pattern with a small
5 number of hybridizations identified as positive which can be investigated further. It is well known in the art to increase stringency of hybridisation gradually until only a few positive clones remain.

As an alternative to probing, though still employing
10 nucleic acid hybridisation, oligonucleotides designed to amplify DNA sequences from *Rht* genes may be used in PCR or other methods involving amplification of nucleic acid, using routine procedures. See for instance "PCR protocols; A Guide to Methods and Applications", Eds. Innis et al, 1990, Academic
15 Press, New York.

Preferred amino acid sequences suitable for use in the design of probes or PCR primers are sequences conserved (completely, substantially or partly) between *Rht* genes.

On the basis of amino acid sequence information,
20 oligonucleotide probes or primers may be designed, taking into account the degeneracy of the genetic code, and, where appropriate, codon usage of the organism from the candidate nucleic acid is derived. In particular, primers and probes may be designed using information on conversed sequences
25 apparent from, for example, Figure 3 and/or Figure 4.

Where a full-length encoding nucleic acid molecule has not been obtained, a smaller molecule representing part of the full molecule, may be used to obtain full-length clones.

Inserts may be prepared for example from partial cDNA clones and used to screen cDNA libraries. The full-length clones isolated may be subcloned into vectors such as expression vectors or vectors suitable for transformation into plants.

5 Overlapping clones may be used to provide a full-length sequence.

The present invention also extends to nucleic acid encoding *Rht* or a homologue obtainable using a nucleotide sequence derived from Figure 2 or Figure 3a, and such nucleic

10 acid obtainable using one or more, e.g. a pair, of primers including a sequence shown in Figure 5.

Also included within the scope of the present invention are nucleic acid molecules which encode amino acid sequences which are homologues of the polypeptide encoded by *Rht* of

15 *Triticum*. A homologue may be from a species other than *Triticum*.

Homology may be at the nucleotide sequence and/or amino acid sequence level. Preferably, the nucleic acid and/or amino acid sequence shares homology with the sequence encoded

20 by the nucleotide sequence of Figure 3a, preferably at least about 50%, or 60%, or 70%, or 80% homology, most preferably at least 90%, 92%, 95% or 97% homology. Nucleic acid encoding such a polypeptide may preferably share with the *Triticum Rht* gene the ability to confer a particular phenotype on

25 expression in a plant, preferably a phenotype which is GA responsive (i.e. there is a change in a characteristic of the plant on treatment with GA), such as the ability to inhibit plant growth where the inhibition is antagonised by GA. As

noted, *Rht* expression in a plant may affect one or more other characteristics of the plant. A preferred characteristic that may be shared with the *Triticum Rht* gene is the ability to complement a *Rht* null mutant phenotype in a plant such as

5 *Triticum*, such phenotype being resistance to the dwarfing effect of paclobutrazol. The *slender* mutant of barley maps to a location in the barley genome equivalent to that of *Rht* in the wheat genome. Such mutant plants are strongly paclobutrazol resistant. The present inventors believe that

10 the *slender* barley mutant is a null mutant allele of the orthologous gene to wheat *Rht*, allowing for complementation of the barley mutant with the wheat gene. Ability to complement a *slender* mutant in barley may be a characteristic of embodiments of the present invention.

15   Some preferred embodiments of polypeptides according to the present invention (encoded by nucleic acid embodiments according to the present invention) include the 17 amino acid sequence which is underlined in Figure 3b, or a contiguous sequence of amino acids residues with at least about 10

20 residues with similarity or identity with the respective corresponding residue (in terms of position) in 17 amino acids which are underlined in Figure 3b, more preferably 11, 12, 13, 14, 15, 16 or 17 such residues, and/or the sequence DVAQKLEQLE, or a contiguous sequence of amino acids with at

25 least about 5 residues with similarity or identity with the respective corresponding residue (in terms of position) within DVAQKLEQLE, more preferably 6, 7, 8 or 9 such residues. Further embodiments include the 27 amino acid sequence

DELLAALGYKVRASDMADVAQKLEQLE, or a contiguous sequence of amino acids residues with at least about 15 residues with similarity or identity with the respective corresponding residue (in terms of position) within this sequence, more preferably 16,

5   17, 18, 19, 20, 21, 22, 23, 24, 25 or 26 such residues.

As is well-understood, homology at the amino acid level is generally in terms of amino acid similarity or identity. Similarity allows for "conservative variation", i.e. substitution of one hydrophobic residue such as isoleucine,

10   valine, leucine or methionine for another, or the substitution of one polar residue for another, such as arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine. Similarity may be as defined and determined by the TBLASTN program, of Altschul *et al.* (1990) *J. Mol. Biol.* 215: 403-10,

15   which is in standard use in the art. Homology may be over the full-length of the *Rht* sequence of Figure 3b, or may more preferably be over a contiguous sequence of 10 amino acids compared with DVAQKLEQLE, and/or a contiguous sequence of 17 amino acids, compared with the 17 amino acids underlined in

20   Figure 3b, and/or a contiguous sequence of 27 amino acids compared with DELLAALGYKVRASDMADVAQKLEQLE, or a longer sequence, e.g. about 30, 40, 50 or more amino acids, compared with the amino acid sequence of Figure 3b and preferably including the underlined 17 amino acids and/or DVAQKLEQLE.

25   At the nucleic acid level, homology may be over the full-length or more preferably by comparison with the 30 nucleotide coding sequence within the sequence of Figure 3a and encoding the sequence DVAQKLEQLE and/or the 51 nucleotide coding

polypeptide which includes an amino acid sequence which is a mutant, allele, derivative or variant sequence of the *Rht* amino acid sequence of the species *Triticum Aestivum* shown in Figure 3b, or is a homologue of another species or a mutant,

5   allele, derivative or variant thereof, wherein said mutant, allele, derivative, variant or homologue differs from the amino acid sequence shown in Figure 3b by way of insertion, deletion, addition and/or substitution of one or more amino acids, as obtainable by producing transgenic plants by

10 transforming plants which have a *Rht* null mutant phenotype, which phenotype is resistance to the dwarfing effect of paclobutrazol, with test nucleic acid, causing or allowing expression from test nucleic acid within the transgenic plants, screening the transgenic plants for those exhibiting

15 complementation of the *Rht* null mutant phenotype to identify test nucleic acid able to complement the *Rht* null mutant, deleting from nucleic acid so identified as being able to complement the *Rht* null mutant a nucleotide sequence encoding the 17 amino acid sequence underlined in Figure 3b or a

20 contiguous 17 amino acid sequence in which at least 10 residues have similarity or identity with the respective amino acid in the corresponding position in the 17 amino acid sequence underlined in Figure 3b, more preferably 11, 12, 13, 14, 15, 16 or 17, and/or a nucleotide sequence encoding

25 DVAQKLEQLE or a contiguous sequence of 10 amino aicds with at least about 5 (more preferably 6, 7, 8 or 9) which have similarity or identity with the corresponding residue in the sequence DVAQKLEQLE.

A cell containing nucleic acid of the present invention represents a further aspect of the invention, particularly a plant cell, or a bacterial cell.

The cell may comprise the nucleic acid encoding the
5 enzyme by virtue of introduction into the cell or an ancestor thereof of the nucleic acid, e.g. by transformation using any suitable technique available to those skilled in the art.

Also according to the invention there is provided a plant cell having incorporated into its genome nucleic acid as
10 disclosed.

The present invention also provides a plant comprising such a plant cell.

Also according to the invention there is provided a plant cell having incorporated into its genome a sequence of
15 nucleotides as provided by the present invention, under operative control of a regulatory sequence for control of expression. A further aspect of the present invention provides a method of making such a plant cell involving introduction of a vector comprising the sequence of
20 nucleotides into a plant cell and causing or allowing recombination between the vector and the plant cell genome to introduce the sequence of nucleotides into the genome.

A plant according to the present invention may be one which does not breed true in one or more properties. Plant
25 varieties may be excluded, particularly registrable plant varieties according to Plant Breeders' Rights. It is noted that a plant need not be considered a "plant variety" simply because it contains stably within its genome a transgene,

expression, and therefore growth and/or development of the plant according to preference. Furthermore, mutants and derivatives of the wild-type gene may be used in place of the endogenous gene. The inserted gene may be foreign or

5   exogenous to the host cell, e.g. of another plant species.

The principal characteristic which may be altered using the present invention is growth.

According to the model of the *Rht* gene as a growth repressor, under-expression of the gene may be used to promote

10   growth, at least in plants which have only one endogenous gene conferring *Rht* function (not for example *Arabidopsis* which has endogenous homologues which would compensate). This may involve use of anti-sense or sense regulation. Taller plants may be made by knocking out *Rht* or the relevant homologous

15   gene in the plant of interest. Plants may be made which are resistant to compounds which inhibit GA biosynthesis, such as paclobutrazol, for instance to allow use of a GA biosynthesis inhibitor to keep weeds dwarf but let crop plants grow tall.

Over-expression of a *Rht* gene may lead to a dwarf plant

20   which is correctable by treatment with GA, as predicted by the *Rht* repression model.

Since *rht* mutant genes are dominant on phenotype, they may be used to make GA-insensitive dwarf plants. This may be applied for example to any transformable crop-plant, tree or

25   fruit-tree species. It may provide higher yield/reduced lodging like Rht wheat. In rice this may provide GA-insensitive rice resistant to the Bakane disease, which is a problem in Japan and elsewhere. Dwarf ornamentals may be of

value for the horticulture and cut-flower markets. Sequence

manipulation may provide for varying degrees of severity of

dwarfing, GA-insensitive phenotype, allowing tailoring of the

degree of severity to the needs of each crop-plant or the

5   wishes of the manipulator. Over-expression of *rht*-mutant

sequences is potentially the most useful.

A second characteristic that may be altered is plant

development, for instance flowering. In some plants, and in

certain environmental conditions, a GA signal is required for

10   floral induction. For example, GA-deficient mutant

Arabidopsis plants grown under short day conditions will do

not flower unless treated with GA: these plants do flower

normally when grown under long day conditions. *Arabidopsis*

gai mutant plants show delayed flowering under short day

15   conditions: severe mutants may not flower at all. Thus, for

instance by *Rht* or *rht* gene expression or over-expression,

plants may be produced which remain vegetative until given GA

treatment to induce flowering. This may be useful in

horticultural contexts or for spinach, lettuce and other crops

20   where suppression of bolting is desirable.

The nucleic acid according to the invention may be placed

under the control of an externally inducible gene promoter to

place the *Rht* or *rht* coding sequence under the control of the

user.

25   The term "inducible" as applied to a promoter is well

understood by those skilled in the art. In essence,

expression under the control of an inducible promoter is

"switched on" or increased in response to an applied stimulus.

The nature of the stimulus varies between promoters. Some inducible promoters cause little or undetectable levels of expression (or no expression) in the absence of the appropriate stimulus. Other inducible promoters cause

5 detectable constitutive expression in the absence of the stimulus. Whatever the level of expression is in the absence of the stimulus, expression from any inducible promoter is increased in the presence of the correct stimulus. The preferable situation is where the level of expression

10 increases upon application of the relevant stimulus by an amount effective to alter a phenotypic characteristic. Thus an inducible (or "switchable") promoter may be used which causes a basic level of expression in the absence of the stimulus which level is too low to bring about a desired

15 phenotype (and may in fact be zero). Upon application of the stimulus, expression is increased (or switched on) to a level which brings about the desired phenotype.

Suitable promoters include the Cauliflower Mosaic Virus 35S (CaMV 35S) gene promoter that is expressed at a high level

20 in virtually all plant tissues (Benfey et al, 1990a and 1990b); the maize glutathione-S-transferase isoform II (GST-II-27) gene promoter which is activated in response to application of exogenous safener (WO93/01294, ICI Ltd); the cauliflower meri 5 promoter that is expressed in the

25 vegetative apical meristem as well as several well localised positions in the plant body, eg inner phloem, flower primordia, branching points in root and shoot (Medford, 1992; Medford et al, 1991) and the *Arabidopsis thaliana LEAFY*

promoter that is expressed very early in flower development
(Weigel et al, 1992).

The GST-II-27 gene promoter has been shown to be induced
by certain chemical compounds which can be applied to growing
5   plants. The promoter is functional in both monocotyledons and
dicotyledons. It can therefore be used to control gene
expression in a variety of genetically modified plants,
including field crops such as canola, sunflower, tobacco,
sugarbeet, cotton; cereals such as wheat, barley, rice, maize,
10 sorghum; fruit such as tomatoes, mangoes, peaches, apples,
pears, strawberries, bananas, and melons; and vegetables such
as carrot, lettuce, cabbage and onion. The GST-II-27 promoter
is also suitable for use in a variety of tissues, including
roots, leaves, stems and reproductive tissues.
15 Accordingly, the present invention provides in a further
aspect a gene construct comprising an inducible promoter
operatively linked to a nucleotide sequence provided by the
present invention, such as the *Rht* gene of *Triticum* a
homologue from another plant species or any mutant, derivative
20 or allele thereof. This enables control of expression of the
gene. The invention also provides plants transformed with
said gene construct and methods comprising introduction of
such a construct into a plant cell and/or induction of
expression of a construct within a plant cell, by application
25 of a suitable stimulus, an effective exogenous inducer. The
promoter may be the GST-II-27 gene promoter or any other
inducible plant promoter.

When introducing a chosen gene construct into a cell,

following introduction of the nucleic acid into plant cells, optionally followed by regeneration into a plant, e.g. using one or more marker genes such as antibiotic resistance (see above).

5   Plants transformed with the DNA segment containing the sequence may be produced by standard techniques which are already known for the genetic manipulation of plants. DNA can be transformed into plant cells using any suitable technology, such as a disarmed Ti-plasmid vector carried by Agrobacterium

10   exploiting its natural gene transfer ability (EP-A-270355, EP-A-0116718, NAR 12(22) 8711 - 87215 1984), particle or microprojectile bombardment (US 5100792, EP-A-444882, EP-A-434616) microinjection (WO 92/09696, WO 94/00583, EP 331083, EP 175966, Green *et al.* (1987) *Plant Tissue and Cell Culture*,

15   Academic Press), electroporation (EP 290395, WO 8706614 Gelvin Debeyser - see attached) other forms of direct DNA uptake (DE 4005152, WO 9012096, US 4684611), liposome mediated DNA uptake (e.g. Freeman *et al. Plant Cell Physiol.* 29: 1353 (1984)), or the vortexing method (e.g. Kindle, *PNAS U.S.A.* 87: 1228

20   (1990d). Physical methods for the transformation of plant cells are reviewed in Oard, 1991, *Biotech. Adv.* 9: 1-11.

       Agrobacterium transformation is widely used by those skilled in the art to transform dicotyledonous species. Recently, there has been substantial progress towards the

25   routine production of stable, fertile transgenic plants in almost all economically relevant monocot plants (Toriyama, et al. (1988) *Bio/Technology* 6, 1072-1074; Zhang, *et al.* (1988) *Plant Cell Rep.* 7, 379-384; Zhang, *et al.* (1988) *Theor Appl*

Genet 76, 835-840; Shimamoto, *et al.* (1989) *Nature* 338, 274-276; Datta, *et al.* (1990) *Bio/Technology* 8, 736-740; Christou, *et al.* (1991) *Bio/Technology* 9, 957-962; Peng, *et al.* (1991) International Rice Research Institute, Manila, Philippines

5   563-574; Cao, *et al.* (1992) *Plant Cell Rep.* 11, 585-591; Li, *et al.* (1993) *Plant Cell Rep.* 12, 250-255; Rathore, *et al.* (1993) *Plant Molecular Biology* 21, 871-884; Fromm, *et al.* (1990) *Bio/Technology* 8, 833-839; Gordon-Kamm, *et al.* (1990) *Plant Cell* 2, 603-618; D'Halluin, *et al.* (1992) *Plant Cell* 4,

10   1495-1505; Walters, *et al.* (1992) *Plant Molecular Biology* 18, 189-200; Koziel, *et al.* (1993) *Biotechnology* 11, 194-200; Vasil, I. K. (1994) *Plant Molecular Biology* 25, 925-937; Weeks, *et al.* (1993) *Plant Physiology* 102, 1077-1084; Somers, *et al.* (1992) *Bio/Technology* 10, 1589-1594; WO92/14828). In

15   particular, *Agrobacterium* mediated transformation is now emerging also as an highly efficient transformation method in monocots (Hiei *et al.* (1994) *The Plant Journal* 6, 271-282).

The generation of fertile transgenic plants has been achieved in the cereals rice, maize, wheat, oat, and barley

20   (reviewed in Shimamoto, K. (1994) *Current Opinion in Biotechnology* 5, 158-162.; Vasil, *et al.* (1992) *Bio/Technology* 10, 667-674; Vain et al., 1995, *Biotechnology Advances* 13 (4): 653-671; Vasil, 1996, *Nature Biotechnology* 14 page 702).

Microprojectile bombardment, electroporation and direct

25   DNA uptake are preferred where Agrobacterium is inefficient or ineffective. Alternatively, a combination of different techniques may be employed to enhance the efficiency of the transformation process, eg bombardment with Agrobacterium

coated microparticles (EP-A-486234) or microprojectile bombardment to induce wounding followed by co-cultivation with Agrobacterium (EP-A-486233).

Brassica napus transformation is described in Moloney *et*
5 *al.* (1989) *Plant Cell Reports* 8: 238-242.

Following transformation, a plant may be regenerated, e.g. from single cells, callus tissue or leaf discs, as is standard in the art. Almost any plant can be entirely regenerated from cells, tissues and organs of the plant.
10 Available techniques are reviewd in Vasil et al., *Cell Culture and Somatic Cel Genetics of Plants, Vol I, II and III, Laboratory Procedures and Their Applications*, Academic Press, 1984, and Weissbach and Weissbach, *Methods for Plant Molecular Biology*, Academic Press, 1989.

15 The particular choice of a transformation technology will be determined by its efficiency to transform certain plant species as well as the experience and preference of the person practising the invention with a particular methodology of choice. It will be apparent to the skilled person that the
20 particular choice of a transformation system to introduce nucleic acid into plant cells is not essential to or a limitation of the invention, nor is the choice of technique for plant regeneration.

In the present invention, over-expression may be achieved
25 by introduction of the nucleotide sequence in a sense orientation. Thus, the present invention provides a method of influencing a characteristic of a plant, the method comprising causing or allowing expression of nucleic acid according to

of protein from the target gene occurs. When the inserted

gene is only part of the endogenous gene the number of

under-expressing individuals in the transgenic population

increases. The mechanism by which sense regulation occurs,

5   particularly down-regulation, is not well-understood.

However, this technique is also well-reported in scientific

and patent literature and is used routinely for gene control.

See, for example, See, for example, van der Krol *et al.*,

(1990) *The Plant Cell* 2, 291-299; Napoli *et al.*, (1990) *The*

10   *Plant Cell* 2, 279-289; Zhang *et al.*, (1992) *The Plant Cell* 4,

1575-1588, and US-A-5,231,020.

Thus, the present invention also provides a method of

influencing a characteristic of a plant, the method comprising

causing or allowing expression from nucleic acid according to

15   the invention within cells of the plant. This may be used to

influence growth.

Aspects and embodiments of the present invention will now

be illustrated, by way of example, with reference to the

20   accompanying figures. Further aspects and embodiments will be

apparent to those skilled in the art. All documents mentioned

in this text are incorporated herein by reference.

The following Figures are included herein:

25   Figure 1: Alignment of N-terminus predicted GAI amino

acid sequence (Gai) with rice EST D39460 (0830), with a region

of homology outlined in black.

Figure 2: DNA sequences from C15-1, 14a1 and 5a1.

Figure 2a shows a consensus DNA sequence cDNA C15-1 (obtained via single-pass sequencing).

Figure 2b shows data from original DNA sequencing runs from 14a1 (single-pass).

5   Figure 2c shows data from original DNA sequencing runs from 5a1 (single-pass).

Figure 3:   Rht sequences.

Figure 3a shows a composite DNA sequence of wheat *Rht* gene derived from data in Figure 2, including coding sequence.

10   Figure 3b shows an alignment of the entire predicted Rht protein sequence encoded by the coding sequence of Figure 2 (rht) with the entire predicted GAI protein sequence of *Arabidopsis* (Gai). Regions of sequence identity are highlighted in black.

15   Figure 4:   D39460 sequence.

Figure 4a shows DNA sequence (single-pass) of rice cDNA D39460. This cDNA is an incomplete, partial clone, missing the 3' end of the mRNA from which it is derived.

Figure 4b shows alignment of the entire predicted Rht
20 protein sequence (wheat - encoded by the coding sequence of Figure 2) with that of GAI (Gai) and rice protein sequence predicted from DNA sequence in Figure 4a (Rice). Regions of amino acid identity are highlighted in black; some conservative substitutions are shaded.

25   Figure 5:   Primers used for sequencing Rht clones (Figure 2).

Figure 6:   The basic carbon-ring structure of gibberellins.

Previously, we cloned the *GAI* gene of *Arabidopsis* (PCT/GB97/00390). Comparison of the DNA sequences of the wild-type (*GAI*) and mutant (*gai*) alleles showed that *gai* encodes a mutant predicted protein product (gai) which lacks a

5 segment of 17 amino acids from close to the N-terminus of the protein. Screening of the DNA sequence databases with the *GAI* sequence revealed the existence of a rice EST (D39460) which contains a region of sequence very closely related to that of the segment that is deleted from GAI in the gai protein. A

10 comprison of the predicted amino acid sequences from the region DELLA to EQLE are identical in both sequences. The two differences (V/A; E/D) are conservative substitutions, in which one amino acid residue is replaced by another having very similar chemical properties. In addition, the region of

15 identity extends beyond the boundary of the deletion region in the gai protein. The sequence DVAQKLEQLE is not affected by the deletion in gai, and yet is perfectly conserved between the GAI and D39460 sequences (Figure 1).

D39460 was used in low-stringency hybridization

20 experiments to isolate hybridizing clones from wheat cDNA and genomic libraries. Several clones were isolated, including C15-1 and C15-10 (cDNAs), and 5al and 14al (genomic clones). Clone C15-1 has been used in gene mapping experiments. Nullisomic-tetrasomic analysis showed that clone C15-1

25 hybridizes to genomic DNA fragments derived from wheat chromosomes 4A, 4B and 4D. This is consistent with clone C15-1 containing *Rht* sequence, since the *Rht* loci map to the gorup 4 chromosomes. Furthermore, recombinant analysis using a

population segregating for the *Rht-D1b* (formerly *Rht2*) allele

identified a hybridizing fragment that displayed perfect co-

segregation with the mutant allele. This placed the genomic

location of the gene encoding the mRNA sequence in cDNA C15-1

5   within a 2 cM segment (that was already known to contain *Rht*)

of the group 4 chromosomes, and provides strong evidence that

the cDNA and genomic clones do indeed contain the *Rht* gene.

Figure 2a gives the complete (single-pass) DNA sequence

of cDNA C15-1. We have also obtained DNA sequence for C15-10;

10   it is identical with that of C15-1, and is therefore not

shown. Figures 2b and 2c show original data from individual

sequencing runs from clones 14a1 and 5a1. The sequences shown

in Figure 2 can be overlapped to make a composite DNA

sequence, shown in Figure 3a. This sequence displays strong

15   homology with that of *Arabidopsis GAI*, as revealed by a

comparison of the amino acid sequence of a predicted

translational product of the wheat sequence (Rht) with that of

GAI (GAI), shown in Figure 3b. In particular, the predicted

amino acid sequence of the presumptive *Rht* reveals a region

20   of near-identity with GAI over the region that is missing in

gai (Figure 4). Figure 4 reveals that the homology that

extends beyond the gai deletion region in the rice EST is also

conserved in Rht (DVAQKLEQLE), thus indicating that this

region, in addition to that found in the gai deletion, is

25   involved in GA signal-transduction. This region is not found

in SCR, another protein that is related in sequence to GAI but

which is not involved in GA signalling. The primers used in

the above sequencing experiments are shown in Figure 5.

```
                                                                    54
                                                                    60

Gai  ......MKRD HHHHHQDKKT MMNEEDDGN GMDELLAVLG YKVRSSEMAD VAQKLEQLEV  54
803  EAGGSSGGGS SADMGSCKDK VMAGAxGEEE xVDELLAALG YKVRSSDMAD VAQKLEOLEM  60

Gai  MSNVQEDDL  SQLATETVHY NPAELYTWLD
803  AMGMGGVTPP AORMTGSCRT WPRTKFI..
```

Figure 1

2

Consensus sequence of c-15-1

Cdna.Se   Length: 1746 August 7, 1997 09:08 Type: N Check: 4797 ..

```
   1  CCCCGACGGT CGCGGCCGCG GCCAACGCGA CGCCCGCGCT GCCGGTCGTC

  51  GTGGTCGACA CGCAGGAGGC CGGGATTCGG CTGGTGCACG CGCTGCTGGC

 101  GTGCGCGGAG GCCGTGCAGC AGGAGAACCT CTCCGCCGCG GAGGCGCTGG

 151  TGAAGCAGAT ACCCTTGCTG GCCGCGTCCC AGGGCGGCGC GATGCGCAAG

 201  GTCGCCGCCT ACTTCGGCGA GGCCCTCGCC CGCCGCGTCT TCCGCTTCCG

 251  CCCGCAGCCG GACAGCTCCC TCCTCGACGC CGCCTTCGCC GACCTCCTCC

 301  ACGCGCACTT CTACGAGTCC TGCCCCTACC TCAAGTTCGC GCACTTCACC

 351  GCCAACCAGG CCATCCTGGA GGCGTTCGCC GGCTGCCGCC GCGTGCACGT

 401  CGTCGACTTC GGCATCAAGC AGGGGATGCA GTGGCCCGCA CTTCTCCAGG

 451  CCCTCGCCCT CCGTCCCGGC GGCCCTCCCT CGTTCCGCCT CACCGGCGTC

 501  GGCCCCCCGC AGCCGGACGA GACCGACGCC CTGCAGCAGG TGGGCTGGAA

 551  GCTCGCCCAG TTCGCGCACA CCATCCGCGT CGACTTCCAG TACCGCGGCC

 601  TCGTCGCCGC CACGCTCGCG GACCTGGAGC CGTTCATGCT GCAGCCGGAG

 651  GGCGAGGAGG ACCCGAACGA AGAXCCCGAX GTAATCGCCG TCAACTCAGT

 701  CTTCGAGATG CACCGGCTGC TCGCGCAGCC CGGCGCCCTG GAAAAGGTTC

 751  TTGGGCACCG TGCGCCCCCG TGCGGCCCAG AATTCXTCAC CGTGGTGGAA

 801  ACAGGAGGCA AATCACAACT CCGGCACATT CCTGGACCGC TTCACCGAGT

 851  CTCTGCACTA CTACTCCACC ATGTTCGATT CCCTCGAGGG CGGCAGCTCC

 901  GGCGGCGGCC CATCCGAAGT CTCATCGGGG GCTGCTGCTG CTCCTGCCGC

 951  CGCCGGCACG GACCAGGTCA TXTCCGAGGT GTACCTCGGC CGGCAGATCT

1001  GCAACGTGGT GGCCTGCGAG GGGGCGGAAC GCACAGAXCG CCACGAGACG

1051  CTGGGCCAGT GGCGGAACCG GCTGGGCAAC GCCGGGTTCG AGACCGTCCA

1101  CCTGGGCTCC AATGCCTACA AGCAGGCGAX CACGCTGCTG GCGCTCTTCG

1151  CCGGCGGCGA ACGGCTACAX GTGGAAGAAA AGGAAGGCTG CCTGACGCTG

1201  GGGTTGCACA CXCCCCCCTG ATTGCCACCT CGGCATGGCG CCTGGCCGGG

1251  CCGTGATCTC GCGAGTTTTG AACGCTGTAA GTACACATCG TGAGCATGGA

1301  GGACAACACA GCCCCGGCGG CCGCCCCGGC TCTCCGGCGA ACGCACGCAC

1351  GCACGCACTT GAAGAAGAAG AAGCTAAATG TCATGTCAGT GAGCGCTGAA

1401  TTGCAGCGAC CGGCTACGAT CGATCGGGCT ACGGGTGGTT CCGTCCGTCT

1451  GGCGTGAAGA GGTGGATGGA CGACGAACTC CGAGCCGACC ACCACCGGCA

1501  TGTAGTAATG TAATCCCTTC TTCGTTCCCA GTTCTCCACC GCCTCCATGA
```

```
15:   TCACCCGTAA AACTCCTAAG CCCTATTATT ACTACTATTA TGTTTAAATG

1601  TCTATTATTG CTATGTGTAA TTCCTCCAAC CGCTCATATC AAAATAAGCA

1651  CGGGCCGGAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA

1701  AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAA
```

```
  1  CGCGCAATGC TTAAGGTCXC CGCCTACTTC GGXGCAGGCC CTCGCCCGCC

 51  GCGTCTTCCG CTTCCGCCCG CAGCCGGACA GCTCCCTCCT CGACGCCGCC

101  TTCGCCGACC TCCTCCACGC GCACTTCTAC XAGTCCTGCC CCTACCTCAA

151  GTTCGCGCAC TTCACCGCCA ATTAGGCCAT CCTGGAGGCG TTCGCCGGCT

201  GCCGCCGCGT GCACGTCGTC GACTTCGGCA TCAAGCAGGG GATGCAGTGG

251  CCCGCACTTC TCCAGGCCCT CGCCCTCCGT CCCGGCGGCC CTCCCTCGTT

301  CCGCCTCACC GGCGTCGGCC CCCCGCAGCC GG
```

14 a 1 clone Sequenced
with Rha-1 primer

Figure 26-②

5

```
  1   ACCTCCTTCG TCGTCTXTXX GGTGGGGGCG CCAGGAGCTT ATGTGGTGGA

 51   GGXTGGCCCC XCCGGTCGCG ACCGCGXCCT ACGXGACGCC CGCGCTGCCG

101   GTCGTCGTGG TCGACACGCA GGAGGCCGGG ATTCGGXTGG TXCACGCGCT

151   GCTGGXGTGC GXGGAGXCCG TGCAGCAGGA GAACCTCTCC GCCGCGGAGG

201   CGCTXGTGAA GXAGATACCC XTGCTGGCCG AGTCCCAGGG CGGCGAGATG

251   XGCAAGGTXG CAGCTTACTT XGXAGAXGCC CTCGCCCGCX GAGTGATTCC

301   ACTTAXCGCC TGCAGCCGGA XAGCTCCGTC CTCGAAXCCG CXTTXGCCGA

351   CCTCCTCCAC GXGCACXTXT ACGAGTC
```

14a1 clone Sequenced
with Rha-2 Primer

6

```
  1  TAXTAGTCTC TCGGTGGGGG CGCCAGGAGC TCTXTGGTGG AGGCXGCCCC

 51  GCCGGTCGCG GCCGCGGCCA ACGCGACGCC CGCGCTGCCG GTCGTCGTGG

101  TCGACACGCA GGAGGCCGGG ATTCGGATGG TGCACGCGCT GXTGGCGTGC

151  GCGGAGGCCG TGAAACAGTT GAAGGXCCXC GCCTXXXXXC XCACAAXXTG

201  AAAGCCCCGX G
```

14 a 1 clone Sequenced
with Rha-3 Primer

Fig 2 b -④

```
  1  GGCTXCCXCC XCGTGCACGT CGTCGACTTC GGCATCAAGC ATGGGATGCA
 51  XTGGCXCGXA CTTCTCCAXG CCCTCGCCCT CCGTCCCGGC GGCCCTCCCT
101  CGTTCCGCCT CACCGGCGTC GGCCCCCCGC AGCCGGACGA GACCGACGCC
151  CTGCAXCAGG TGGGCTGGAA GCTCGCCCAG TTCGCGCACA CCATCCGCGT
201  CGACTTCCAX TACCGTGGCC TCGTCGCCGC CACGCTCGCG GACCTGGAGC
251  CGTTCATGCT GCAXCCGGAG GGCGAGGAGG ACCCGAACGA CGGAGCCCGA
301  GGTAATCGCC GTCAACTCAG TCTTCGAGAT GCACCGGGCT GCTCXCGCAX
351  CCCGGCGACX CTGGAAXAA
```

14 a 1 clone Sequenced
with Rha-5 Primer

Fig 2 b-(5)

```
  1   CAAGAXGCTA ATCACAACTC CGGCACATTC CTGGACCGCT TCACCGAGTC

 51   TCTGCAXTAC TACTCCACCA TGTTCGATTC CCTCGAGGGC GGCAGCTCCG

101   GCGGCGGCCC ATCCGAAGTC TCATCGGGGG CTGCTGCTGC TCCTGCCGCC

151   GCCGGCACGG ACCATGTCAT GTCCGAXGTG TACCTCGGCC GGCAGATCTG

201   CAACGTGGTG GCCTGCGAGG GGGCGGAGCG CACAXTAXCG CCACGCAGAC

251   XCTGGGCCAG TGGCGTGAAC CGGCTGGGCA ACGCCXGGTT CAXXXXCCGT

301   CCACCTGGGC TCCAATGCCT ACAATCAXGC XXXCACGCTG CTGGCGCCTC

351   TTCGCCC
```

14 a 1 clone Sequenced
with Rha-? Primer

Figure 20-b

```
  1  TCGCCAXTCG GCATGGXGCC TGGCCGGGCC GTGATCTCGC GAGTTTTGAA

 51  CGCTGTAAGT ACACATCGTG AGCATGGAGG ACAACACAGC CCCGGCGGCC

101  GCCCCGGCTC TCCGGCGAAC GCACGCACGC ACGCACTTGG AAGAAGAAXA

151  AGCTAAATGT CATGTCAGTG AGCGCTGAAT TGCAACGACC GGCTACGATC

201  GATCGGGCTA CGGGTGGTTC CGTCCGTCTG GCGTGAAGAG GTGGATGGAC

251  GACGAACTCC GAXCCGACCA CCACCGGCAT GTAGTAATGT AATCCCTTCT

301  TCGTTCCCAG TTCTCCACCG CCTCCATGGA TCACCCGTAA AACTCCTAAG

351  CCCTAATTAT XXACTAACTA ATTATGTTTT AAAATGTTCT AATTAATTGG

401  CTATGTTGTA ATXCCTCCAA ACCGGCTCAT TTTCAAAXAT TAAGCCACGG

451  GCCCGGAACT TTGGTTTAAC AACCTCCCXA TTGXAAAATT XAAATXGAAA

501  TTTTTGGTTX C
```

14 a 1 clone Sequenced
with Rha-8 primer

```
  1   GTTGGTGGXG GCGATTTGGG TACAAGGTGC GCGCCTCCGA CATGGXGGAX

 51   GTGGGGCAGA AGCTGGAGCA GXTCGAGATG GCCATGGGGA TGGGXGGCGT

101   GGGCGCTGGC GCCGCCCCTG ACGACAGGTT XGCCACCCGC XGGCCGCGGA

151   CACXGTGCAX TACAACCCCA CXGACXTGTC GTCTTGGGTC GAGAGCATGC

201   TGTCGGAGCT AAAXGAGCCG CXGCCGCCCC TCCCGCCCGC CCCGCAGCTC

251   AACGCCTCCA CCTCCTCCAC CGTCACGGGC AGCGGCGGCT ACTTCGATAA

301   CCCTCCCTG
```

14 a 1 clone Sequenced
with Rht-9 primer

```
  1   TGATGGXGGG AGXTTAXGGG TTAXAAATGT GGGGGAXTTC CGAAXXGGTG

 51   AGGAXATATX XTCAGAAGTT GGAGCAGATG AGAGATXGCT GATGGGGATA

101   GGGTAGGXGT GGGTGCCGGT GCXGCCCCX AGGAXAGATT GGCCACCCAC

151   TTAGCAAGTG GAXACCGTGG ATTACXACCC CACAGACCTG TCGTGGTTGG

201   GTTTGAGAGC GTGGTGTGGG AGCTGAACGG GCXGCGGCGT GCCCCTCCCG

251   CCCGCCCCGC AGCTCAACGC CTCCACCTCC TCCACCGTAC ACGGGCAGCG

301   GCGGCTAGTT CGATCTCCCG CCCTCCGTCG ACTCCTCCAG CAGCATXTAX

351   GCGCTGCGGC CGATCCCCTX CCCAAGCXXG CGXGGXCCGA GCCGTGTAX
```

14 a 1 clone Sequenced
with Rht-10 primer

12

```
  1   TTTCAXTTTC XTCCTTTTTT CTTCTTTTTC CAACCCCCGG CCCCCXGACC

 51   CTTGGATCCA AATCCCGAAC CCGCCCCCAG AACCXGGAAC CGAGGCCAAG

101   CAAAAGXTTT GCGCCAATTA TTGGCCAGAG ATAGATAGAG AGGCGAGGTA

151   GCTCGCGGAT CATGAAGCGG GAGTACCAGG ACGCCGGAGG GAGCGGCGGC

201   GGCGGTGGCG GCATGGGTTC GTCCGAGGAC AAGATGATGG TGTCGGCGGC

251   GGCGGGGGAG GGGGAGGAGG TGGACGAGCT GCTGGCGGCG CTCGGGTACA

301   AGGTGCGCGC CTCCGACATG GCGGACGTGG CGCAGAAGCT GGAGCAGCTC

351   GAGATGGCCA TGGGGATGGG CGGCGTGGGC GCCGGCGCCG CCCCCGACGA

401   CAGCTTCGCC ACCCACCTCG CCACGGACAC CGTGCAGTAC AACCXCCCXG

451   ACC
```

14 a 1 clone Sequenced
with Rht-12 Primer

Figure 2 b-(10)

13

```
  1   GGACGACGAC CTCCGAGCCG ACCACCACCG GCATGTAGTA ATGTAATCCC

 51   TTCTTCXTTC CCAGTXCTCC ACCGCCTCCA TGATCACCCG TAAAACTCCT

101   AAGCCCTATT ATTACTACTA TTATGTXTAA XTGTCTATTA TTGCTAXGTG

151   TAATTCCTCC AACCGCTCAT ATCAAAATAA GCACGGGCCG GACTTTGTTA

201   XCAGCTCCAA TGAGAATGAA ATGAATTTTG TACGCAAGGC ACGTCCAAAA

251   CTGGGCTGAG CTTTGTTCTG TTCTGTTATG TTCATGGTGC TCACTGCTCT

301   GATGAACATG ATGGTGCCTC CAATGGTGGC TTTGCAATTG TTGAAACGTT

351   TGGCTTGGGG GACTTGXGTG GGTGGGTGCA TGGGGATGAA TATTCACATC

401   XCCGGATTAA AATTAAGCCA TCCCGTTGGC CGTCCTTTGA ATAXCTTGCC

451   CXAAACGAAA TTTCCCCCXA TC
```

14a1 clone Sequenced
with Rht-13 primer

Figure 26-⑪

14

```
  1  AAAXCCTAXA AXATATAGAG GCGATGTXGC XCCCCXATCA XXAACXGGAT

 51  TACXGXAACX CCXGAAGGAG CGGCGGCGGC GGTGGCAGCA TXGGCTCGTC

101  CGATGACAAA TATCATGGTG TCGGCGGCGG CGGGGGACGG GGAGGAGGTG

151  CACAACXTTT XGGCGGGACT CGXGTACCAC GTGXACGGTG CCGCXCTXGX

201  GGATXTGGCC CTXGAAGATG GGCCACCTCC AAA
```

14a1 clone Sequenced
with Rht-14 Primer

Figure 2 b - (12)

15

```
1    CGGCGGCCCC GTGGCGGCAT GGGCTCGTCC GAGGACXAGA TGATGGTGTC

51   GGCGGCGGCG GGGGAXGGGG ATGATGTGGA CTATCTGCTG GCGGCGCTCG

101  GGTACAAGGT GCGCGCCTCC GACAGGCGGA GCCCGCGCAT AACTGGAGCC

151  GCTCGAGATG GCCXTGGGGA TXGGCGGCXT GGGCXCCXGC GCCTCCCCCG
```

14 a 1 clone Sequenced
with Rht-15 primer

16

```
  1   TGGXGCTCGG GTGXCCCGTG CGCGCCTCCG ACATGGCGGG ACGTGGCGCA

 51   GAACTGGAGC AGCTCGAGAT GGCCATGGGG ATGGGCGGCG TGGGCGCCGG

101   CGCCGCCCCC GACGACAGCT TCGCCACCCA CCTCGCCACG GACACCGGCA

151   CACAACCCCA CCGACCTGTC GTCTTGGGTC GAGAGCATGC TGTCGGATCT

201   CXACGCGCCX CCGXCGCCCC TCCCGCCCGC
```

14a1 clone Sequenced
with Rht-16 Primer

```
     AXXTTGTXCX XXXTACATCC CATGXCCGC GCXATGCTXA AGGTCGCCGC
51   CTACTTCGGC GCAGGCCCTC GCCCGCCGCG TCTTCCGCTT CCGCCCGCAG
101  CCGGACAGCT CCCTCCTCGA CGCCGCCTTC GCCGACCTCC TCCACGCGCA
151  CTTCTACGAG TCCTGCCCCT ACCTCAAGTT CGCGCACTTC ACCGCCAACC
201  AGGCCATCCT GGAGGCGTTC GCCGGCTGCC GCCGCGTGCA CGTCGTCGAC
251  TTCGGCATCA AGCAGGGGAT GCAGTGGCCC GCACTTCTCC AGGCCCTCGC
301  CCTCCGTCCC GGCGGCCCTC CCTCGTTCCG CCTCACCGGC GTTCGGCCCC
351  CCGCAGCCGG ACGAXAACGA CGCCCTG
```

5a1 clone Sequenced with
Rha-1 primer

18

```
  1   XTTCCCCGGC AGTTAAAAGC XTCCACTTCT TCCACCGTCA CGGGCAGCGG

 51   CGGXTACTTX GATCTCCCGC CCTCAGTCGA CTCCTCCAGC AGCATCTACG

101   CGCTGCGGCC GATCCCCTCC CCGGCCGGCG CGACGGCGCC GGCCGACCTG

151   TCCGCCGACT CCGTGCGGGA TCCCAAGCGG ATGCGCACTG GCGGGAGCAG

201   CACCTCGTCG TCATCCTCCT CATAXTCGTC TCTCGGTGGG GGCGCCAGGA

251   GCTCTGTGGT GGAGGCXGCC CCGCCGGTCG CGGCCGCGGC CAACGCGACG

301   CCCGCGCTGC CGGTCGTCGT GGTCGACACG CAGGAGGCCG GGATTCGGAT

351   GGTGCACGCG CTGXTGGCGT GCGCGGAGGC CGTGXAAGCA GTTXGAAGGG

401   CCTXCGCCGT GXATXXCGCA ACAAXXXGGA AGXCCX
```

Sa1 clone Sequenced with Rha-3 primer

Figure 2 C - (3)

```
  1   CAXCCCGCTG XTCGCCACCT CGGCATGGCG CCTGGCCGGG CCGTGATCTC

 51   GCGAGTTTTG AACGCTGTAA GTACACATCG TGAGCATGGA GGACAACACA

101   GCCCCGGCGG CGGCCCCGGC TCTCCGGCGA ACGCACGCAC GCACGCACTT

151   GAAGAAGAAG AAGCTAAATG TCATGTCAGT GAGCGCTGAA TTGCAXCGAC

201   CGGCTACGAT CGATCGGGCT ACGGGTGGTT CCGTCCGTCT GGCGTGAAGA

251   GGTGGATGGA CGACGAACTC CGAXCCGACC ACCACCGGCA TGTAGTAATG

301   TAATCCCTTC TTCGTTCCCA GTTTCTCCAC CGCCTCCATG ATCACCCCGT

351   AAAACTCCTA AGCCCTATXX XTTACTACXA TTAATGTTTT AAAXTGTTCT

401   AXTAATTGCT ATGXTGTTTA TTXCC
```

5a1 clone Sequenced
with Rha-8 primer

Figure 2 c - ④

20

```
  1   TATCGAAGTA GCCGCCGCTG CCCXTGCACG GTGGAGGAGG TGGAGGCGTT

 51   GAGCTGCGGG GCGGGCGGGA GGGGCGGCGG CGGCACGTTX AGCTCCGACA

101   GCATGCTCTC GACCCAAAAC XACAGGTCGG TGGGGTTGTA GTGCACGGTG

151   TCCGTGGCGA GGGGGTGGCX AAXCTGTCGT CAGGGGCGGC GCCXGCGCCC

201   ACXCCGCCCA TCCCCATGGC CATCTCGAXC TGCTCCAGCT TCTGCGCCAC

251   TTCCXCCATG TCXGATGCGC GCXCCTTGTA CCCGA
```

Sal clone Sequenced
with Rht-9 primer

Figure 2 G-⑤

21

```
  1  ACGGCGCGGX CCXCGCXXGC TTGGGAGGGG ATCGGCCGCA GCGCXTAXAT

 51  GCTGCTGGAG GAGTCGACGG AGGGCGGGAG ATCGAACTAG CCGCCGCTGC

101  CCGTGTACGG TGGAGGAGGT GGAGGCGTTG AGCTGCGGGG CGGGCGGGAG

151  GGGCAGCXGC TGCACGTTXA GCTCCCACAC CACGTCTCTC AACCCAACCA

201  CGACXCGTCT GTGGGGTXGT AATXCACGGT XTCCCTXGCT AXGTGGGTGG

251  CCAATCTXT
```

Sa1 clone sequenced
with Rht-10 primer

Figure 2C-6

```
  1   CACGGTGTCC GTGGCGAGGT GGGTGGCGAA GCTGTCGTCG GGGGCGGCGC

 51   CGGCGCCCAC GCCGCCCATC CCCATGGCCA TCTCGAGCTG CTCCAGCTTC

101   TGCGCCACGT CCGCCATGTC GGAGGCGCGC ACCTTGTACC CGAGCGCCGC

151   CAGCAGCXCG XCCACCTCCT CCCCCTCCCC CGCCGCCGCC GACACCATCA

201   TCTTGTCCTC GGACGAXCCC ATGCCGCCAC CGCCGCCGCC GCTCCCTCCG

251   GCGTCCTGGT ACTCCCGCTT CATGATCCGC GAGCTACCTC GCCTCTCTAT

301   CTATCTCTGG CCAATAATTG CGCA
```

5a1 clone Sequenced
with Rht-12 primer

Figure 2 C-(4)

```
  1   GACCACCACC GGCATGTAGT AATGTAATCC CTTCTTCXTT CCCAGTTCTC

 51   CACCGCCTCC ATGATCACCC GTAAAACTCC TAAGCCCTAT TATTACTACT

101   ATTATGTXTA AATGTCTATT ATTGCTAXGT GTAATTCCTC CAACCGCTCA

151   TATCAAAATA AGCACGGGCC GGACTTTGTT AGCAGCTCCA ATGAGAATGA

201   AATGAATTTT GTACGCAAGG CACGTCCAAA ACTGGGCTGA GCTTTGTTCT

251   GTTCTGTTAT GTTCATGGTG CTCACTGCTC TGATGAACAT GATGGTGCCT

301   CCAATGGGTG GCTTTGCAAT TGTTGAACGT TTTGGCTTGG GGGACTTGGT

351   GXXTGGTGCA TGGGAATGAA XATTCCACAT CCXCXGGAAT TAAAATTAGC

401   CCATCCCG
```

5a1 clone Sequenced
with Primer Rht-13

Figure 3 a

```
   1   TTTCAXTTTC XTCCTTTTTT CTTCTTTTTC CAACCCCCGG CCCCCXGACC

  51   CTTGGATCCA AATCCCGAAC CCGCCCCCAG AACCXGGAAC CGAGGCCAAG

 101   CAAAAGXTTT GCGCCAATTA TTGGCCAGAG ATAGATAGAG AGGCGAGGTA

 151   GCTCGCGGAT CATGAAGCGG GAGTACCAGG ACGCCGGAGG GAGCGGCGGC

 201   GGCGGTGGCG GCATGGGTTC GTCCGAGGAC AAGATGATGG TGTCGGCGGC

 251   GGCGGGGGAG GGGGAGGAGG TGGACGAGCT GCTGGCGGCG CTCGGGTACA

 301   AGGTGCGCGC CTCCGACATG GCGGACGTGG CGCAGAAGCT GGAGCAGCTC

 351   GAGATGGCCA TGGGGATGGG CGGCGTGGGC GCTGGCGCCG CCCCTGACGA

 401   CAGGTTXGCC ACCCGCXGGC CGCGGACACX GTGCAXTACA ACCCCACXGA

 451   CXTGTCGTCT TGGGTCGAGA GCATGCTGTC GGAGCTAAAX GAGCCGCXGC

 501   CGCCCCTCCC GCCCGCCCCG CAGCTCAACG CCTCCACCGT CACGGGCAGC

 551   GGCGGXTACT TXGATCTCCC GCCCTCAGTC GACTCCTCCA GCAGCATCTA

 601   CGCGCTGCGG CCGATCCCCT CCCCGGCCGG CGCGACGGCG CCGGCCGACC

 651   TGTCCGCCGA CTCCGTGCGG GATCCCAAGC GGATGCGCAC TGGCGGGAGC

 701   AGCACCTCGT CGTCATCCTC CTCATAXTCG TCTCTCGGTG GGGGCGCCAG

 751   GAGCTCTGTG GTGGAGGCXG CCCCGCCGGT CGCGGCCGCG GCCAACGCGA

 801   CGCCCGCGCT GCCGGTCGTC GTGGTCGACA CGCAGGAGGC CGGGATTCGG

 851   CTGGTGCACG CGCTGCTGGC GTGCGCGGAG GCCGTGCAGC AGGAGAACCT

 901   CTCCGCCGCG GAGGCGCTGG TGAAGCAGAT ACCCTTGCTG GCCGCGTCCC

 951   AGGGCGGCGC GATGCGCAAG GTCGCCGCCT ACTTCGGCGA GGCCCTCGCC

1001   CGCCGCGTCT TCCGCTTCCG CCCGCAGCCG GACAGCTCCC TCCTCGACGC

1051   CGCCTTCGCC GACCTCCTCC ACGCGCACTT CTACGAGTCC TGCCCCTACC

1101   TCAAGTTCGC GCACTTCACC GCCAACCAGG CCATCCTGGA GGCGTTCGCC

1151   GGCTGCCGCC GCGTGCACGT CGTCGACTTC GGCATCAAGC AGGGGATGCA

1201   GTGGCCCGCA CTTCTCCAGG CCCTCGCCCT CCGTCCCGGC GGCCCTCCCT

1251   CGTTCCGCCT CACCGGCGTC GGCCCCCCGC AGCCGGACGA GACCGACGCC

1301   CTGCAGCAGG TGGGCTGGAA GCTCGCCCAG TTCGCGCACA CCATCCGCGT

1351   CGACTTCCAG TACCGCGGCC TCGTCGCCGC CACGCTCGCG GACCTGGAGC

1401   CGTTCATGCT GCAGCCGGAG GGCGAGGAGG ACCCGAACGA AGAXCCCGAX

1451   GTAATCGCCG TCAACTCAGT CTTCGAGATG CACCGGCTGC TCGCGCAGCC
```

```
15r   CGGCGCCCTG GAAAAGGTTC TTGGGCACCG TGCGCCCCCG TGCGGCCCAG

1551  AATTCXTCAC CGTGGTGGAA ACAGGAGGCA AATCACAACT CCGGCACATT

1601  CCTGGACCGC TTCACCGAGT CTCTGCACTA CTACTCCACC ATGTTCGATT

1651  CCCTCGAGGG CGGCAGCTCC GGCGGCGGCC CATCCGAAGT CTCATCGGGG

1701  GCTGCTGCTG CTCCTGCCGC CGCCGGCACG GACCAGGTCA TXTCCGAGGT

1751  GTACCTCGGC CGGCAGATCT GCAACGTGGT GGCCTGCGAG GGGGCGGAAC

1801  GCACAGAXCG CCACGAGACG CTGGGCCAGT GGCGGAACCG GCTGGGCAAC

1851  GCCGGGTTCG AGACCGTCCA CCTGGGCTCC AATGCCTACA AGCAGGCGAX

1901  CACGCTGCTG GCGCTCTTCG CCGGCGGCGA ACGGCTACAX GTGGAAGAAA

1951  AGGAAGGCTG CCTGACGCTG GGGTTGCACA CXCCCCCCTG ATTGCCACCT

2001  CGGCATGGCG CCTGGCCGGG CCGTGATCTC GCGAGTTTTG AACGCTGTAA

2051  GTACACATCG TGAGCATGGA GGACAACACA GCCCCGGCGG CCGCCCCGGC .

2101  TCTCCGGCGA ACGCACGCAC GCACGCACTT GAAGAAGAAG AAGCTAAATG

2151  TCATGTCAGT GAGCGCTGAA TTGCAGCGAC CGGCTACGAT CGATCGGGCT

2201  ACGGGTGGTT CCGTCCGTCT GGCGTGAAGA GGTGGATGGA CGACGAACTC

2251  CGAGCCGACC ACCACCGGCA TGTAGTAATG TAATCCCTTC TTCGTTCCCA

2301  GTTCTCCACC GCCTCCATGA TCACCCGTAA AACTCCTAAG CCCTATTATT

2351  ACTACTATTA TGTTTAAATG TCTATTATTG CTATGTGTAA TTCCTCCAAC

2401  CGCTCATATC AAAATAAGCA CGGGCCGGAC TTTGTTAXCA GCTCCAATGA

2451  GAATGAAATG AATTTTGTAC GCAAGGCACG TCCAAAACTG GGCTGAGCTT

2501  TGTTCTGTTC TGTTATGTTC ATGGTGCTCA CTGCTCTGAT GAACATGATG

2551  GTGCCTCCAA TGGTGGCTTT GCAATTGTTG AAACGTTTGG CTTGGGGGAC

2601  TTGXGTGGGT GGGTGCATGG GGATGAATAT TCACATCXCC GGATTAAAAT

2651  TAAGCCATCC CGTTGGCCGT CCTTTGAATA XCTTGCCCXA AACGAAATTT

2701  CCCCCXATC
```

Figure 3b

```
PRETTYBOX  of: My.Msf(*)   August 7, 1997 13:0?:42.76

Gai  ......M    KRDHHHHQ.   ......D     KKTMMNEED   DGNGMDELLA  VLGYKVRSSE   41
Rht  IERRGSSRIM KREYQDAGGS  GGGGGMGSE   DKMMVSAAAG  EGEEVDELLA  ALGYKVRASD   60

Gai  MADVAQKLEQ LEVMMS....  ..NVQEDD    LSQLATETVH  YNPAELYTWL  DSMLTDLNPP   93
Rht  MADVAQKLEQ LEMAMGMGGV  GAGAAPDRQV  XHPXAADTVX  YNPTDXSSWV  ESMLSELXEP   120

Gai  XPPLPPAPQL NASTVTGSGG  ..GAGA      SNAEYDLKAI  PGDAILNQFA  IDSASSSNQ.   123
Rht  .......... SSSSSXSSL   ........    SSIYALRPI   PSPAGATAPA  DLSADSVRDP   180

Gai  ...GGGGDT  YTTNKRLKCS  NG...VVE    ..TTTAT     AESTRHVVLV  DSQENGVRLV   169
Rht  KRMRTGGSST SSSSSXSSL   GGARSSVVE   AAPPVAAAAN  ATPALPVVVV  DTQEAGIRLV   240

Gai  HALLACAEAV QKENLTVAEA  SQIGAMRKVA  TYFAEALARR  ........    ..........   227
Rht  HALLACAEAV QQENLSAAEA  SQGGAMRKVA  AYFGEALARR  ........    ..........   300

Gai  SPIDHSLSDT LQMHFYETCP  QAILEAFQGK  KRVHVIDFSM  ........    SQGLQWPALM   287
Rht  SLLDAAFADL LHAHFYESCP  QAILEAFAGC  RRVHVDFEGI  ........    KQGMQWPALL   360

Gai  QALALRPGGP PVFRLTGIGP  EVGCKLAHLA  EAIHVEFEYR  GFVANTLADL  ..........   347
Rht  QALALRPGGP PSFRLTGVGP  QVGWKLAQFA  HTIRVDFQYR  GLVAATLADL  ..........   420

Gai  DASMLELRPS EIES...     AVNSVFELHK  LLGRPGAIDK  VLG.VVNQIK  PEIFTVVE.Q   400
Rht  EPFMLQPEGE EDPNEXPXVI  AVNSVFEMHR  LLAQPGALEK  VLGHRAPPCG  PEFXTVVETQ   480

Gai  ESNHNSPIFL DRFTESLHYY  STLFDSLEGV  PSGQ..      .DKVMSEVY   LLALFNGGEG   442
Rht  EANHNSGTFL DRFTESLHYY  STMFDSLEGG  SSSGGGPSEVS GTDQVXSEVY  LLALFAGGER   540

Gai  LGKQICNVVA CDGPDRVERH  ETLSQWRNRF  .SAGFAAAHI  GSNAFKQASM  ..........   502
Rht  LGRQICNVVA CEGAERTXRH  ETLGQWRNRL  GNAGFETVHL  GSNAYKQAXT  ..........   600

Gai  YRVEESDGCL MLGWHTRPLI  ATSAWKLSTN  532
Rht  LXVEEKEGCL TLGLHTXPLI  ATSAWRLAGP  630
```

Figure 4a

```
  1   ACGCGTCCGG AAGCCGGCGG GAGCAGCGGC GGCGGGAGCA GCGCCGATAT

 51   GGGGTCGTGC AAGGACAAGG TGATGGCGGG GGCGGCGGGG GAGGAGGAGG

101   ACGTCTACGA GCTGCTGGCG GCGCTCGGGT ACAAGGTGCG GTCGTCCGAC

151   ATGGCCGACG TCGCGCAGAA XCTGGAGCAG CTGGAGATGG CCATGGGGAT

201   GGGCGGCGTG AGCGCCCCCG GCGCCGCGGA TGACGGGTTC GTGTCGCACC

251   TGGCCACGGA CACCGTGCAC TACAACCCCT CGGACCTCTC CTCCTGGGTT

301   CXGAGAGCAT GCTTTCGGAG TTAAAGGCGC CGTTGCCCCT TATCCCGCCA

351   GGCGCCGCCG GGCTGCCCGC CATGCTTTCC AACTTCGTCC ACTGTCACCG

401   GCGGCGGTGG TAGCGGCTTC TTTGAAXTCC CAGCCGCTGC CGAXTCGTCG ;

451   AGTAGCACXT ACGCCCTCAG GCCGATCTCC TTACCGGTGG TGGCGACGGC

501   TGACCCGTCG GCTGCTGACT CGGCGAGGGA CACCAAGCGG ATGCGCACTG

551   GCGGCGGCAG CACGTCGTCG TCCTCATCGT CGTCTTCCTC TCTGGGCGGT

601   GGGGCCTCGC GGGGCTCTGT GGTGGAGGCT GCTCCGCCGG CGACGCAAGG

651   GGCCGCGGCG GCGAATGCGC CCGCCGTGCC GGTTGTGGTG GTTGACACGC

701   AGGAGGCTGG XATCGGGCCT GGTGC
```

Figure - 46

PRETTYB□ of: My.Msf(*)   August 7, 1997 12:0^:41.67

```
Wheat  IERRGSSRIM KREYQDAGGS GGGGGMGSE  DKMMVSAAAG EGEEVDELLA ALGYKVRASD  60
Rice   .........M TRPEAGGSSG GGSSADMGSC KDKVMAGAAG EEEDVDELLA ALGYKVRSSD  50
Gai    .........M KRDHHHHHQD ..........  KKTMMNEED  DGNGMDELLA VLGYKVRSSE  41

Wheat  MADVAQKLEQ LEMAMGMGGV GAGAAPDRQV XHPXAADTVX ESMLSELXEP            120
Rice   MADVAQxLEQ LEMAMGMGGV SAPGAADDGF VSHLATDTVH ESMLSELKAP            110
Gai    MADVAQKLEQ LEVMMS.... ...NVQEDD  LSQLATETVH DSMLTDLNPP             93

Wheat  XPPLPP.APQ LNA....S   TVTGSG.GY  XDLPPSVDSS SSIYALRPIP SPAGATAPAD 171
Rice   LPLIPPGAAG LPAMLSPTSS TVTGGGGSF  FExPAAAxSS SSTYALRPIS LPVVATADPS 170
Gai    ........   .......    ......GY   FEx.....SS NAEYDLKAIP GDAILN...Q 112

Wheat  LSADSVRDPK RMRTGGSSTS SSSSSXSSLG GGA.RSSVVE AAPPV..AAA ANATPALPVV 228
Rice   .AADSARDTK RMRTGGGSTS SSSSSXSSLG GGASRGSVVE AAPPATQGAA AANAPAVPVV 229
Gai    FAIDSA....              SSSNQGGGG DTYTNKRLK  CSNGVVETTT ATAESTRHVV 157

Wheat  VVDTQ..EAG IRLVHALLAC AEAVQQENLS AAEALVKQIP LLAASQGGAM RKVAAYFGEA 286
Rice   VVDTQ.EEAG IRLVHALLAC XEAVQQENER ..........  ..........  ..........  258
Gai    LVDSQ..ENG VRLVHALLAC AEAVQKENLT VAEALVKQIG FLAVSQIGAM RKVATYFAEA 215

Wheat  LARRVFRFRP QPDSSLLDAA FADLLHAHFY ESCPYLKFAH FTANQAILEA FAGCRRVHVV 346
Rice   ..........  ..........  ..........  ..........  ..........  ..........  258
Gai    LARRIYRLSP SQ..SPIDHS LSDTLQMHFY ETCPYLKFAH FTANQAILEA FQGKKRVHVI 273

Wheat  DFGIKQGMQM PALLQALALR PGGPPSFRLT GVGPPQPDET DALQQVGWKL AQFAHTIRVD 406
Rice   ..........  ..........  ..........  ..........  ..........  ..........  258
Gai    DFSMSQGLQW PALMQALALR PGGPPVFRLT GIGPPAPDNF DYLHEVGCKL AHLAEAIHVE 333

Wheat  FQYRGLVAAT LADLEPFMLQ PEGEEDPNEX PXVIAVNSVF EMHRLLAQPG ALEKVLGHRA 466
Rice   ..........  ..........  ..........  ..........  ..........  ..........  258
Gai    FEYRGFVANT LADLDASMLE LRPSEIES.. ...VAVNSVF ELHKLLGRPG AIDKVLG.VV 387

Wheat  PPCGPEFXTV VETQEANHNS GTFLDRFTES LEGGSSGGGP SEVSSGAAAA            526
Rice   NQIKPEIFTV VE.QESNHNS PIFLDRFTES LEGVPSGQ.. ..........            258
Gai    ..........  ..........  ..........  ..........  ..........            434

Wheat  PAAAGTDQVX SEVYLGRQIC NVVACEGAER TXRHETLGQM RNRLGNAGFE TVHLGSNAYK 586
Rice   ..........  ..........  ..........  ..........  ..........  ..........  258
Gai    ....DKVM   SEVYLGKQIC NVVACDGPDR VERHETLSQM RNRFGSAGFA AAHIGSNAFK 488
```

figure 46 (cont'

29

PRETTYBOX of: My.Msf(*)   August 7, 1997 12:04:41.67

```
Wheat   QAXTLLALFA GGERLXVEEK EGCLTLGLHT XPLIATSAWR LAGP630
Rice    ....      GGEGYRVEES DGCLMLGWHT RPLIATSAWK LSTN .258
Gai     QASMLLALFN                                       .532
```

Figure 3

Primers used in the RHT sequencing project.

| Name	Sequence	Sense
RHA-1	CTG GTG AAG CAG ATA CCC TTG C	Forward
RHA-2	CTG GTT GGC GGT GAA GTG CG	Reverse
RHA-3	GCA AGG GTA TCT GCT TCA CCA GC	Reverse
RHA-5	CGC ACT TCA CCG CCA ACC AG	Forward
RHA-7	CCG TGC GCC CCC GTG CGG CCC AG	Forward
RHA-8	AGG CTG CCT GAC GCT GGG GTT GC	Forward
RHT-9	GAT CGG CCG CAG CGC GTA GAT GC	Reverse
RHT-10	GAT CCC GCA CGG AGT CGG CGG ACA G	Reverse
RHT-12	TCC GAC AGC ATG CTC TCG ACC CAA G	Reverse
RHT-13	TTC CGT CCG TCT GGC GTG AAG AGG	Forward
RHT-14	AAA TCC CGA ACC CGC CCC CAG AAC	Forward
RHT-15	GCG CCA ATT ATT GGC CAG AGA TAG	Forward
RHT-16	GGC ATG GGT TCG TCC GAG GAC AAG	Forward
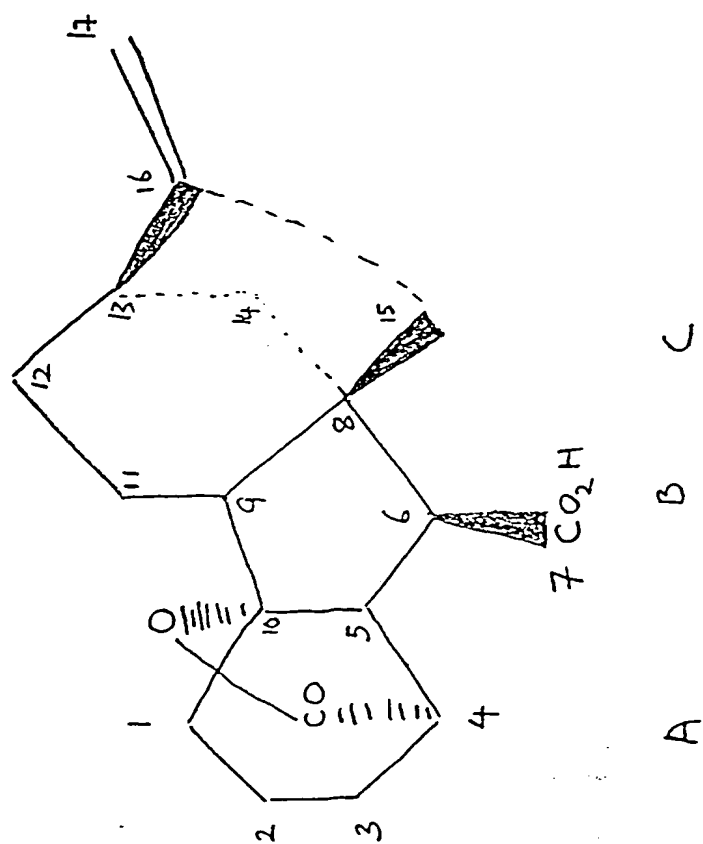
Figure 6

① 98102383.

② 7 aug 08.

③. melbourn Ellis.